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(54) **Method for the production of a polypeptide, RNA or other compound in tumor tissue**

(57) Described is a method for the production of a polypeptide, RNA or other compound comprising (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor and (b) isolating the desired polypeptide, RNA or compound from the tumor tissue. This method is also useful for the concomitant production of a desired antigen and an antibody directed to said antigen, wherein said method comprises the additional step of isolating antibodies from the serum of the animal bearing the tumor that produces the antigen.

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Description

[0001] The present invention relates to a method for the production of a polypeptide, RNA or other compound comprising (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor and (b) isolating the desired polypeptide, RNA or compound from the tumor tissue. The method of the invention is also useful for the concomitant production of a desired antigen and an antibody directed to said antigen, wherein said method comprises the additional step of isolating antibodies from the serum of the animal bearing the tumor that produces the antigen.

[0002] Until now, the engineering and large scale production of recombinant proteins, e.g., in tissue cultures, has been time consuming and expensive, prohibiting in many cases the wide spread use of these proteins, e.g., in medicine. Recent developments in protein engineering and production technologies have contributed to overcome at least some of these problems. For example, using molecular farming, i.e. the production of recombinant proteins in transgenic plants can be used to synthesize foreign proteins, e.g., antibodies and vaccines on an agricultural scale. However, this approach also has some drawbacks, e.g., in the case of recombinantly produced human proteins the glycosylation pattern of the protein differs from the pattern of the natural protein.

[0003] Therefore, it is the object of the present invention to provide a means for the efficient recombinant production of biologically active proteins which overcomes disadvantages of the approaches for recombinant production of proteins presently used.

[0004] The solution to the above technical problem has been achieved by providing the embodiments characterized in the claims. In EP-A1 1 281 772 it is diclosed that when vaccinia virus (LIVP strain) carrying the light emitting fusion gene construct rVV-ruc-gfp was injected intravenously into nude mice, the virus particles were found to be cleared from all internal organs within 4 days, as determined by extinction of light emission. In contrast, when the fate of the injected vaccinia virus was similarly followed in nude mice bearing tumors grown from subcutaneously implanted C6 rat glioma cells, virus particles were found to be retained over time in the tumor tissues, resulting in lasting light emission. The presence and amplification of the virus-encoded fusion proteins in the same tumor were monitored in live animals by observing GFP fluorescence under a stereomicroscope and by collecting luciferase-catalyzed light emission under a low-light video-imaging camera. Tumor-specific light emission was detected 4 days after viral injection in nude mice carrying subcutaneous C6 glioma implants. Tumor accumulation of rVV-ruc-gfp virus particles was also seen in nude mice carrying subcutaneous tumors developed from implanted PC-3 human prostate cells, and in mice with orthotopically implanted MCF-7 human breast tumors. Further, intracranial C6 rat glioma cell implants in immunocompetent rats and MB-49 human bladder tumor cell implants in C57 mice were also targeted by the vaccinia virus. In addition to primary breast tumors, small metastatic tumors were also detected externally in the contralateral breast region, as well as in nodules on the exposed lung surface, suggesting metastasis to the contralateral breast and lung. In summary, it was shown that light-emitting cells or microorganims, e.g. vaccinia virus can be used to detect and treat primary and metastatic tumors.

[0005] Similar results were obtained with light-emitting bacteria *(Salmonella, Vibrio, Listeria, E. coli)* which were injected intravenously into mice and which could be visualized in whole animals under a low light imager immediately. No light emission was detected twenty-four hours after bacterial injection in both athymic (nu/nu) mice and immunocompetent C57 mice as a result of clearing by the immune system. In nude mice baring tumors developed from implanted C6 glioma cells, light emission was abolished from the animal entirely twenty-four hours after delivery of bacteria, similar to mice without tumors. However, forty-eight hours post-injection, unexpectedly, a strong, rapidly increasing light emission originated only from the tumor regions was observed. This observation indicated a continuous bacterial replication in the tumor tissue. The extent of light emission was dependent on the bacterial strain used. The homing-in process together with the sustained light emission was also demonstrated in nude mice carrying prostate, bladder, and breast tumors. In addition to primary tumors, metastatic tumors could also be visualized as exemplified in the breast tumor model. Tumor-specific light emission was also observed in immunocompetent C57 mice with bladder tumors as well as in Lewis rats with brain glioma implants. Once in the tumor, the light-emitting bacteria were not observed to be released into the circulation and to re-colonize subsequently implanted tumors in the same animal. Further, mammalian cells expressing the Ruc-GFP fusion protein, upon injection into the bloodstream, were also found to home into and propagate in glioma tumors. These findings opened the way for designing multifunctional viral vectors useful for the detection of tumors based on signals like light emission, for suppression of tumor development and angiogenesis signaled by, e.g., light extinction and the development of bacterium- and mammalian cell-based tumor targeting systems in combination with therapeutic gene constructs for the treatment of cancer. These systems have the following advantages: (a) They target the tumor specifically without affecting normal tissue; (b) the expression and secretion of the therapeutic gene constructs are, preferably, under the control of an inducible promoter, enabling secretion to be switched on or off; and (c) the location of the delivery system inside the tumor can be verified by direct visualization before activating gene expression and protein delivery.

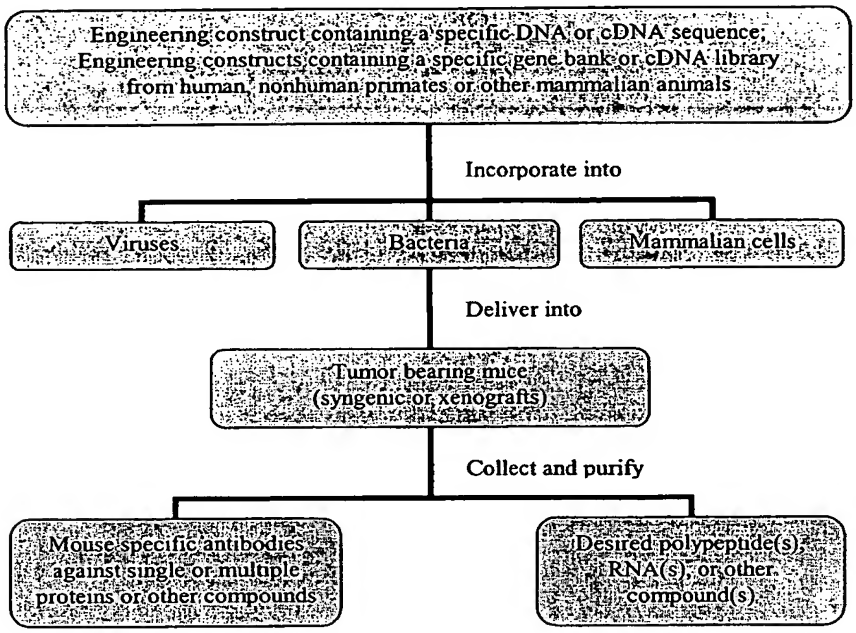
[0006] During the experiments leading to the present invention it has been found that the system described above

being based on the accmulation of bacteria, viruses etc. in tumors can be used for the simple, quick, and inexpensive production of proteins and other biological compounds originating from cloned DNA sequences. It has also been found that this system is useful for the concomitant overproduction of polypeptides, RNA or other biological compounds (in tumor tissue) and antibodies against those compounds (in the serum) of the same animal. It was shown that after intravenous injection, vaccinia virus enters the tumor of an animal and, due to its immunoprivileged state, replicates preferentially in the tumor tissues and thereby overproduces of the inserted gene encoded protein in the tumors. After harvesting the tumor tissues, the localized and overexpressed protein could be isolated by a simple procedure from tumor homogenates. In addition, based on the findings that only 0.2 to 0.3% of the desired proteins produced in the tumor were found in the blood stream of the same animal, a simultaneous vaccination of the mouse and efficient antibody production against the overproduced protein was achieved. Thus, serum from the same mouse (or any other animal) can be harvested and used as mouse-derived antibodies against the proteins or other products overproduced in the tumor.

[0007] The advantages of the system of the present invention are:

(a) No production of a transgenic animal carrying the novel polypeptide ancoding cassette is required;
(b) the tumor system is more efficient than tissue culture;
(c) proteins interfering with animal development and other toxic proteins may be overproduced in tumors without negative effects to the host animal;
(d) the combined system is fast: Within 4 to 6 weeks from cDNA cloning to protein and antisera purification;
(e) the system is relatively inexpensive and can be scaled up easily;
(f) correct protein folding and modifications can be achieved;
(g) high antigenicity may be achieved which is beneficial for better antibody production; and
(h) species-specific-cell-based production of proteins, e.g., in mice, with tumors as fermentors is achieved.

[0008] The system of the present invention can be illustrated by the following scheme:



[0009] Accordingly, in one embodiment, the present invention relates to a method of producing a desired polypeptide, RNA or compound, said method comprising the following steps: (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor; (b) harvesting the tumor tissue from said animal; and (c) isolating the desired polypeptide, RNA or compound from said tumor tissue.

[0010] Th principal steps of the method of the present invention can be summarized as follows (shown for a particual

embodiment, i.e. vaccinia virus additionally containing a gene encoding a light-emitting protein):

(1) Insertion of the desired DNA or cDNA into the vaccinia virus genome;
(2) modification of the vaccinia virus genome with light-emitting protein construct as expression marker;
(3) recombination and virus assembly in cell culture;
(4) screening of individual viral particles carrying inserts followed by large scale virus production and concentration;
(5) injection of the viral particles into mice or other animals bearing tumors of human, non-human primate or other mammalian origins;
(6) verification of viral replication and protein overproduction in animals based on light emission;
(7) harvest of tumor tissues and, optionally, the blood (separately); and
(8) purification of overexpressed proteins from tumors and, optionally, antisera from blood using conventional methods.

[0011] As used herein, the term "compound" refers to any compound which is produced in the tumor due to the presence of the recombinant polypeptide, e.g., a compound which is generated by the recombinant polypeptide (e.g., enzyme) and the cellular machinery of the tumor.

[0012] Any microorganism or cell is useful for the method of the present invention, provided that they replicate in the animal, are not pathogenic for the animal e.g. attenuated and, are recognized by the immune system of the animal, etc. Suitable micoroganisms and cells are, e.g., disclosed in EP-A1 1 281 772 and EP-A1 1 281 767.

[0013] The person skilled in the art also knows how to generate animals carrying the desired tumor (see, e.g., EP-A1 1 281 767 or EP-A1 1 281 777). The tumor tissue is surgically removed from the animal. After homogenisation of the tumor tissue, the desired polypeptide, RNA or other biological compound can be purified according to established methods. For example, in the case of a recombinant polypeptide, the polypeptide might contain a his-tag and can be purified via column chromatography. The time necessary for accumulation of sufficient amounts of the polypetide etc. in the tumor of the animal depends on many factors, e.g., the kind of animal, kind of tumor etc. and can be determined by the skilled person by routine experimentation. In general, the desired polypeptide expression can be detected two days after virus injection. The expression peaks approximately two weeks after injection, and lasts up to two months.

[0014] In another embodiment, the present invention relates to a method of producing a desired antibody, said method comprising the following steps: (a) injecting a microorganism or cell containing a DNA sequence encoding an antigen into an animal bearing a tumor; and (b) isolating the antibody directed to said antigen from the serum obtained from said animal. The antibodies directed to said antigen can be isolated and purified according to well known methods. Antibodies that are directed against specific contaminating antigens (e.g. bacteria antigens) can be removed by adsorption, and the antibodies directed against the target antigen can be separated from contaminating antibodies by affinity purification, e.g., by immuno affinity chromatography using the recombinant antigen as the ligand of the column.

[0015] In a further emobidment, the present invention relates to a method of simultaneously producing a desired polypeptide, RNA or compound and an antibody directed to said polypeptide, RNA or compound, said method comprising the following steps: (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor; (b) harvesting the tumor tissue from said animal; (c) isolating the desired polypeptide, RNA or compound from said tumor tissue; and (d) isolating the antibody directed to said polypeptide, RNA or compound from the serum obtained from said animal. This approach is particularly useful for generating polypeptides and/or antibodies against the polypeptides which are toxic or unstable, or which require species specific cellular environment for correct folding or modifications.

[0016] In a preferred embodiment of the method of the present invention, said microorganism or cell furthermore contains a DNA sequence encoding a detectable protein, preferably a luminescent or fluorescent protein, or a protein capable of inducing a detectable signal. As used herein, the term "DNA sequence encoding a luminescent and/or fluorescent protein" also comprises a DNA sequence encoding a luminescent and fluorescent protein as fusion protein.

[0017] Preferably, for transfecting the microorgaisms or cells the DNA sequences encoding the desired polypeptide etc. and, optionally, a DNA sequence encoding a detectable protein, preferably a luminescent or fluorescent protein, or a protein capable of inducing a detectable signal, the DNA sequences are present in a vector or an expression vector. A person skilled in the art is familiar with examples thereof. The DNA sequences can also be contained in a recombinant virus containing appropriate expression cassettes. Suitable viruses that may be used in the method of the present invention include baculovirus, vaccinia, sindbis virus, Sendai virus, adenovirus, an AAV virus or a parvovirus, such as MVM or H-1. The vector may also be a retrovirus, such as MoMULV, MoMuLV, HaMuSV, MuMTV, RSV or GaLV. For expression in mammalian cells, a suitable promoter is e.g. human cytomegalovirus "immediate early promoter" (pCMV). Furthermore, tissue and/or organ specific promoters are useful. Preferably, the DNA sequences are operatively linked with a promoter allowing high expression. Such promoters, e.g. inducible promoters, are well-known to the person skilled in the art.

[0018] For generating the above described DNA sequences and for constructing expression vectors or viruses which

contain said DNA sequences, it is possible to use general methods known in the art. These methods include e.g. in vitro recombination techniques, synthetic methods and in vivo recombination methods as described in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, for example. Methods of transfecting cells, of phenotypically selecting transfectants and of expressing the DNA sequences by using the above described vectors are known in the art.

[0019] The person skilled in the art knows DNA sequences encoding luminescent or fluorescent proteins that can be used in the method of the present invention. During the past decade, the identification and isolation of structural genes encoding light-emitting proteins from bacterial luciferase from Vibrio *harveyi* (Belas et al., Science 218 (1982), 791-793) and from *Vibrio fischerii* (Foran and Brown, Nucleic acids Res. 16 (1988), 177), firefly luciferase (de Wet et al., Mol. Cell. Biol. 7 (1987), 725-737), aequorin from *Aequorea victoria* (Prasher et al., Biochem. 26 (1987), 1326-1332), *Renilla* luciferase from *Renilla reniformis* (Lorenz et al., PNAS USA 88 (1991), 4438-4442) and green fluorescent protein from *Aequorea victoria* (Prasher et al., Gene 111 (1987), 229-233) have been described that allow the tracing of bacteria or viruses based on light emission. Transformation and expression of these genes in bacteria allows detection of bacterial colonies with the aid of the low light imaging camera or individual bacteria under the fluorescent microscope (Engebrecht et al., Science 227 (1985), 1345-1347; Legocki et al., PNAS 83 (1986), 9080-9084; Chalfie et al., Science 263 (1994), 802-805).

[0020] Luciferase genes have been expressed in a variety of organisms. Promoter activation based on light emission, using *lux AB* fused to the nitrogenase promoter, was demonstrated in Rhizobia residing within the cytoplasm of cells of infected root nodules by low light imaging (Legocki et al., PNAS 83 (1986), 9080-9084; O'Kane et al., J. Plant Mol. Biol. 10 (1988), 387-399). Fusion of the *lux A* and *lux B* genes resulted in a fully functional luciferase protein (Escher et al., PNAS 86 (1989), 6528-6532). This fusion gene *(Fab2)* was introduced into *Bacillus subtilis* and *Bacillus megatherium* under the xylose promoter and then fed into insect larvae and was injected into the hemolymph of worms. Imaging of light emission was conducted using a low light video camera. The movement and localization of pathogenic bacteria in transgenic arabidopsis plants, which carry the pathogen-activated PAL promoter-bacterial luciferase fusion gene construct, was demonstrated by localizing *Pseudomonas* or *Ervinia spp.* infection under the low light imager as well as in tomato plant and stacks of potatoes (Giacomin and Szalay, Plant Sci. 116 (1996), 59-72).

[0021] All of the luciferases expressed in bacteria require exogenously added substrates such as decanal or coelenterazine for light emission. In contrast, while visualization of GFP fluorescence does not require a substrate, an excitation light source is needed. More recently, the gene cluster encoding the bacterial luciferase and the proteins for providing decanal within the cell, which includes *luxCDABE* was isolated from *Xenorhabdus luminescens* (Meighen and Szittner, J. Bacteriol. 174 (1992), 5371-5381) and *Photobacterium leiognathi* (Lee et al., Eur. J. Biochem. 201 (1991), 161-167) and transferred into bacteria resulting in continuous light emission independent of exogenously added substrate (Fernandez-Pinas and Wolk, Gene 150 (1994), 169-174). Bacteria containing the complete *lux* operon sequence, when injected intraperitoneally, intramuscularly, or intravenously, allowed the visualization and localization of bacteria in live mice indicating that the luciferase light emission can penetrate the tissues and can be detected externally (Contag et al., Mol. Microbiol. 18 (1995), 593-603).

[0022] Thus, in a further preferred embodiment of the method of the present invention the luminescent or fluorescent protein is a luciferase, green fluorescent protein (GFP) or red fluorescent protein (RFP).

[0023] In a particularly preferred embodiment, the microorganism or cell of the method of the present invention additionally contains a gene encoding a substrate for the luciferase. In an even more preferred embodiment, the microorganism or cell of the method of the present invention contains a *ruc-gfp* expression cassette which contains the *Renilla* luciferase (ruc) and Aequorea gfp cDNA sequences under the control of a strong synthetic early/late (PE/L) promoter of *Vaccinia* or contains the *luxCDABE cassette.*

[0024] Preferably, the microorganism is a bacterium, e.g. attenuated bacterium. Particularly preferred is attenuated *Salmonella thyphimurium,* attenuated *Vibrio cholerae or* attenuated *Listeria monocytogenes* or *E.coli.* Alternatively, viruses such as vaccinia virus, AAV, a retrovirus etc. are also useful for the methods of the present invention. Preferably, the virus is vaccinia virus.

[0025] Preferably, the cell of the method of the present invention is a mammalian cell.

[0026] Any laboratory animal can be used for the method of the present invention, e.g., mice, rats, rabbits, guinea pigs, pigs, sheep and horses, with mice being preferred.

[0027] Preferably, the tumor of the laboratory animal is generated by implanting tumor cells into said animal. Generally speaking, for the production of a desired polypeptide, RNA, or compound, any solid tumor type can be used, with a fast growing tumor type being preferred, e.g. C6 rat glioma or HCT116 human colon carcinoma. For the production of a desired antibody, a relatively slow growing tumor type is preferred, e.g. HT1080 human fibrosarcoma and GI-101A human breast carcinoma. For T-independent antibody production, nu-/nu-mice bearing allogenic tumor or xenografts can be used; while for T-dependent antibody production, immuno-competent mice with syngenic tumors may be the choice.

Brief description of the drawing:

[0028] Figure 1 shows the production of Production of Anti β-Galactosidase via Vaccinia Virus Delivered *lac*Z in Tumor Bearing Mice: β-galactosidase from Roche (lane 1), cell lysate from RVGL7 infected CV-1 cells (lane 2), and the tumor lysate from mouse #143, 14 days after infected with RVGL7 (lane 3) were loaded in triplicates and separated in a 10% SDS-polyacrylamide gel. The immuno-blotting was performed with 1:3000 either mouse monoclonal anti β-galactosidase (panel A), or mouse serum taken from either mouse # 116 (panel B) or #119 (panel C). The antigen detection was carried out using Goat Anti Mouse IgG-HRP, 1:3000, and the amplified Opti-4CN Detection Kit.

[0029] The invention is explained by the following example:

Example 1: Production of β-Galactosidase and Anti β-Galactosidase via Vaccinia Virus Delivered lacz in Tumor Bearing Mice

[0030] Thirty five athymic nu/nu mice (5 weeks old, 25g, male) were included in the experiment to study biodistribution and tumor targeting of vaccinia virus (strain LIVP) with different deletions in the genome. Mice were divided into 7 groups with 5 in each group as presented in Table 1.

Table 1.

Experiment Design				
Group	**N of mice**	**Tumor implanted**	**Virus injected**	**Locus-genes inserted**
1	5	None	VGL	wt LIVP
2	5	C6, s.c. $5x10^5$ cells	VGL	wt LIVP
3	5	C6, s.c. $5x10^5$ cells	RVGL1	N-*luc*, *lacZ*
4	5	C6, s.c. $5x10^5$ cells	RVGL5	*HA-lacZ*
5	5	C6, s.c. $5x10^5$ cells	RVGL7	TK-*egfp*, *lacZ*
6	5	C6, s.c. $5x10^5$ cells	RVGL8	NotI-*lacZ*
7	5	C6, s.c. $5x10^5$ cells	RVGL19	TK-*rTrf*, *lacZ*; NotI-RG

[0031] C6 glioma was subcutaneously developed in Group 2 to 7. Five days after tumor cell implantation ($5x10^5$ cells /mouse), each animal was treated with 0.1 ml of viruses at the multiplicity of infection (MOI) $1x10^7$ via tail vein injection. Two weeks after virus injection, all mice were sacrificed and blood samples were collected. Various organs and tumors were also taken from animals for virus titration and β-galactosidase analysis. The β-galactosidase analysis was carried out using Galacto-Light Plus system (Applied Biosystems), a chemiluminescent reporter gene assay system for the detection of β-galactosidase, according to manufactures instructions.

[0032] In non-tumorous mice as well as in tumorous mice injected with wild type vaccinia virus (without reporter genes and without β-galactosidase gene) no β-galactosidase expression could be detected in organs, blood and tumor samples. By contrast, in the tumors of mice infected with β-galactosidase expressing virus, high amounts of β-galactosidase were obtained. β-galactosidase was also detected in blood samples as shown in Table 2, even though there was no virus recovered from the same samples.

Table 2.

Production of β-galactosidase by vaccinia virus in tumor and blood from tumor bearing mice (day 14 after virus injection)					
Group	**Virus injected**	**β-gal in tumor (μg/ mg of total protein)**	**β-gal in serum (μg/mg of total protein)**	**Estimated total β-gal/ tumor (μg)**	**Estimated total β-gal in 5ml blood (μg)**
3	RVGL1	1.59 ± 0.41	$1.38x10^{-2} \pm 1.09x10^{-2}$	489.84	4.00
4	RVGL5	1.51 ± 0.37	$1.16x10^{-2} \pm 1.08x10^{-2}$	330.21	3.62

Table 2. (continued)

Production of β-galactosidase by vaccinia virus in tumor and blood from tumor bearing mice (day 14 after virus injection)					
Group	**Virus injected**	**β-gal in tumor (μg/ mg of total protein)**	**β-gal in serum (μg/mg of total protein)**	**Estimated total β-gal/ tumor (μg)**	**Estimated total β-gal in 5ml blood (μg)**
5	RVGL7	1.35 ± 0.59	$0.95x10^{-2} \pm 1.47x10^{-2}$	616.60	1.83
6	RVGL8	1.81 ± 0.42	$0.86x10^{-2} \pm 0.33x10^{-2}$	962.36	2.38
7	RVGL19	1.30 ± 0.44	$0.26x10^{-2} \pm 0.16x10^{-2}$	463.75	0.60

[0033] To determine if the amount of β-galactosidase presented in mouse blood was sufficient to elicit antibody production, sera taken from two mice (mouse #116 from Group 5, and #119 from Group 6) were selected as the source for primary antibodies against β-galactosidase in Western analysis. β-galactosidase from *E. coli* (Roche, 567 779) was used as the antigen standard, and the mouse monoclonal anti β-galactosidase from *E. coli* (Sigma, G6282) was used as the antibody positive control. The total protein obtained from CV-1 cells 24 hours after infection with RVGL7 at MOI of 1 pfu/cell was used in the Western analysis, along with the tumor protein sample taken from mouse #143, which was also treated with RVGL7.

[0034] The protein samples were prepared in triplicates, each set including the β-galactosidase antigen standard, the cell lysate from RVGL7 infected CV-1 cells, and the tumor lysate from mouse #143. All protein samples were separated in a 10% polyacrylamide gel, and transferred to NitroBind nitrocellulose membrane (MSI) using BioRad semidry blotting system. Immuno-blotting was performed with either 1:3000 mouse monoclonal anti β-galactosidase, or 1:3000 mouse serum taken from either mouse #116 or #119, and 1:3000 Goat AntiMouse IgG-HRP (BioRad). Amplified Opti-4CN Detection Kit (BioRad) was used for antigen detection.

[0035] As shown in Figure 1, mouse sera taken from mouse #116 and #119 give similar results as compared to commercial mouse anti β-galactosidase, indicating both tumor bearing mice produced antibodies against β-galactosidase.

Claims

1. A method of producing a desired polypeptide, RNA or compound, said method comprising the following steps:

   (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor;
   (b) harvesting the tumor tissue from said animal; and
   (c) isolating the desired polypeptide, RNA or compound from said tumor tissue.

2. A method of producing a desired antibody, said method comprising the following steps:

   (a) injecting a microorganism or cell containing a DNA sequence encoding an antigen into an animal bearing a tumor; and
   (b) isolating the antibody directed to said antigen from the serum obtained from said animal.

3. A method of simultaneously producing a desired polypeptide, RNA or compound and an antibody directed to said polypeptide, RNA or compound, said method comprising the following steps:

   (a) injecting a microorganism or cell containing a DNA sequence encoding the desired polypeptide or RNA into an animal bearing a tumor;
   (b) harvesting the tumor tissue from said animal;
   (c) isolating the desired polypeptide, RNA or compound from said tumor tissue; and
   (d) isolating the antibody directed to said polypeptide, RNA or compound from the serum obtained from said animal.

4. The method of any one of claims 1 to 3, wherein said microorganism or cell furthermore contains a DNA sequence encoding a detectable protein or a protein capable of inducing a detectable signal.

5. The method of claim 4, wherein said protein capable of inducing a detectable signal is a luminescent and/or fluorescent protein.

6. The method of claim 5, wherein said luminescent or fluorescent protein is luciferase, RFP or GFP.

7. The method of claim 6, wherein said microorganism or cell additionally contains a gene encoding a substrate for a luciferase.

8. The method of any one of claims 1 to 7, wherein said migroorganism is a bacterium or a virus.

9. The method of claim 8, wherein said virus is vaccinia virus.

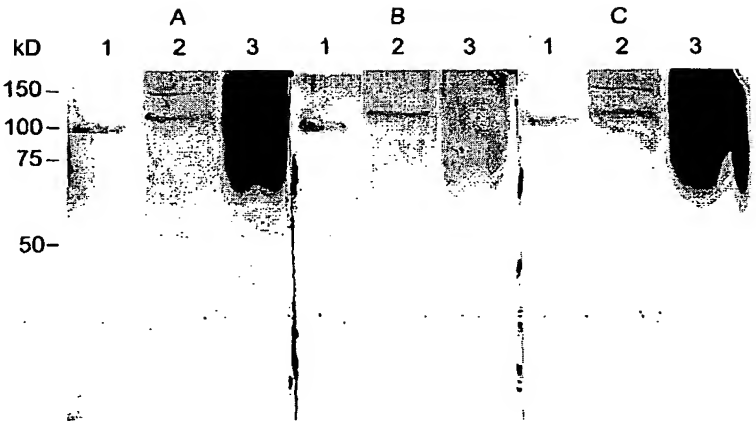
10. The method of claim 9, wherein said bacterium is attenuated *Vibrio cholerae*.

11. The method of any one of claims 1 to 7, wherein said cell is a mammalian cell.

12. The method of any one of claims 1 to 11, wherein siad animal is a mouse.

13. The method of any one of claims 1 to 12, wherein said tumor is a solid tumor.

Figure 1



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 01 8478

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	ZHENG LI-MOU ET AL: "Tumor amplified protein expression therapy: Salmonella as a tumor-selective protein delivery vector" ONCOLOGY RESEARCH, vol. 12, no. 3, 2000, pages 127-135, XP009020983 ISSN: 0965-0407 * page 127, column 1, line 5 - line 14 * * page 128, paragraphs TREATMENT,PROTOCOL * * page 128, paragraphs ENZYMATIC,ASSAY - page 129 * * page 128, paragraphs BACTERIA,QUANTIFICATION * ---	1-13	C12N15/67 C12N15/63 C12N15/85 C07K1/00
X	US 6 589 531 B1 (MCALLISTER-MORENO ANDRES ET AL) 8 July 2003 (2003-07-08) * column 2, line 5 - line 32 * * column 17, line 23-29 * * column 12, line 21 - line 47 * -----	1-13	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

C12N
C07K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	28 November 2003	Mabit, H

CATEGORY OF CITED DOCUMENTS

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 03 01 8478

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